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Fabry disease in female monozygotic twins with complex intronic haplotype variants: a case report

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Abstract

Background Fabry disease is an X-linked lysosomal storage disease caused by the impairment of α-galactosidase A. The complex intronic haplotype (CIH) variants, located in promoter and intronic regulatory lesions, has been found in patients with classical forms of Fabry disease. We present a case of Fabry disease in female monozygotic twins exhibiting the CIH mutation and classical manifestations.

Case presentation A 61-year-old woman with a history of stroke, carotid artery occlusion, hypertrophic cardiomyopathy, and chronic kidney disease was referred to the nephrology clinic for management of her chronic kidney disease. Her monozygotic twin sister also presented with hypertrophic cardiomyopathy, atrial flutter, carotid stenosis, and proteinuria. Clinical symptoms and a comprehensive family history strongly suggested the presence of Fabry disease. Genetic analysis revealed the presence of 5 variants within a complex intronic haplotype (CIH): c.−10 C > T, c.369 + 990 C > A, c.370 − 81_370–77delCAGCC, c.640–16 A > G, and c.1000–22 C > T. We conducted a review of the patient's previous kidney biopsy findings, which demonstrated the presence of lamellated inclusion bodies in electron microscopy. Remarkably, both the monozygotic twin sister and her son exhibited the same genetic mutation. Enzyme replacement therapy was initiated for the patient. Her kidney function decreased throughout a thorough 2-year follow-up period, while there was a slight decrease in the left ventricular mass index.

Conclusions This is the first reported case of female monozygotic twins with the CIH variants representing cardiac, cerebrovascular, and renal manifestations suggestive of Fabry disease.

Keywords Enzyme replacement therapy, Fabry disease, Introns, Twins, Monozygotic, Sequence analysis

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Introduction

Fabry disease is a lysosomal storage disease caused by deficiency or dysfunction of lysosomal hydrolase α-galactosidase A, which is attributed to a mutation in the *GLA* gene. The main characteristics of Fabry disease are due to the accumulation of substances such as globotriaosylceramide (Gb3), globotriaosylsphingosine (lyso-Gb3), and neutral glycosphingolipids within the lysosomes of various tissues. Over 1000 mutations in the *GLA* gene that cause Fabry disease have been identified [1]. In addition, polymorphic *GLA* haplotypes or variants of uncertain significance (VUS), including many *GLA* intron variants, have been reported [2–5]. The accurate interpretation of *GLA* gene variants is imperative for supporting the clinical diagnosis of Fabry disease and their pathogenic role.

Intriguingly, several studies have explored the functional abnormalities of α-galactosidase A enzyme associated with complex intronic haplotype (CIH) mutations [6]. Notably, 5 intronic variants—c−10 C>T within the 5'UTR and exon 1 (rs2071225), c.369+990 C>A within intron 2 (rs1023431), c.370−81delCAGCC within intron 2 (rs5903184), c.640–16 A>G within intron 4 (rs2071397), and c.1000–22 C>T within intron 5 (rs2071228)—have been reported in the context of CIH variants [6]. It is important to recognize that mutations within the intronic regions of the *GLA* gene are linked to a spectrum of clinical manifestations, such as acroparesthesias, hypohidrosis, heat and cold tolerance, gastrointestinal symptoms, left ventricular hypertrophy, proteinuria, and stroke [6–9]. However, conventional gene sequencing techniques focused primarily on exons may overlook intronic *GLA* variants [10]. Consequently, there is the potential for genetic confirmation to be misinterpreted, leading to an underestimation of the prevalence of Fabry disease [11].

Here, we describe a case of monozygotic female twins who exhibited similar clinical symptoms associated with Fabry disease, which was confirmed by identifying CIH mutations using intronic genetic analysis and lamellate lipid inclusions from kidney biopsy.

Case presentation

A 61-year-old woman was referred to our nephrology department for the management of chronic kidney disease. She had a history of stroke, a right internal carotid artery occlusion, hypertrophic cardiomyopathy, chronic kidney disease, and chronic hepatitis B. Her chronic hepatitis B was in an inactive carrier state with no evidence of cirrhosis, and she had not received antiviral treatment. She denied having previously experienced pain or paresthesia at the extremities. Two years previously, a kidney biopsy had been performed to evaluate the cause of proteinuria. At that time, light microscopy revealed basement membrane thickening, mesangial matrix widening with focal mesangial cell proliferation, and moderate interstitial fibrosis and tubular atrophy that was diagnosed as chronic glomerulonephritis. Initially, no abnormal findings were described in the EM of the biopsy. After this, her follow-up was discontinued while being treated for a stroke. At the time of her visit, she complained of intermittent lower abdominal cramping, but she did not exhibit acroparesthesia, hypohidrosis, or angiokeratoma. She has not taken any cationic amphiphilic drugs, such as chlorpromazine, amiodarone or chloroquine, which could have caused phospholipidosis. Initial laboratory tests indicated a serum creatinine level of 2.86 mg/dL, an estimated glomerular filtration rate (eGFR) of 17.18 mL/min/1.73 m^2, and a urinary albumin-to-creatinine ratio of 2790.6 mg/g creatinine. Echocardiography showed hypertrophic cardiomyopathy with a left ventricular mass index of 163.5 g/m^2 (normal range<115 g/m^2) (Fig. 1A and B). Cardiac MRI was not performed. Ophthalmological test did not reveal any abnormal finding. The patient was a monozygotic twin. Her twin sister had histories of atrial flutters, carotid stenosis, proteinuria, left ventricular hypertrophy, and syncope. A family history taking did not reveal any clinical symptoms suggestive of Fabry disease in her parents.

Considering the progression of nephropathy, hypertrophic cardiomyopathy, neurologic symptoms, and her family history with a twin sister, we strongly doubted that the cause was Fabry disease. The α-galactosidase enzyme activity and plasma lyso-Gb3 level were measured, but they were within normal ranges (3.02 μmol/h/L and <1.00 ng/mL, respectively). However, because α-galactosidase enzyme activity could not be decreased in female patients due to random X-chromosome inactivation, we further analyzed a genetic test for the *GLA* gene, including the intronic regions. This analysis revealed the 5 variants of CIH variants within the *GLA* gene (c.–10 C>T (rs2071225), c.369+990 C>A (rs1023431), c.370–81_370–77delCAGCC (rs5903184), c.640–16 A>G (rs2071397), and c.1000–22 C>T (rs2071228)). Therefore, we reviewed the previous kidney biopsy tissue. Electron microscopy showed lamellated lipid inclusions in podocytes, which were compatible with Fabry disease (Fig. 2). Genetic testing of her family confirmed that her monozygotic twin sister and son had the same CIH variants in their *GLA* genes (Fig. 3). The α-galactosidase enzyme activity twin sister and son were within normal range (5.58 μmol/h/L and 4.92 μmol/h/L, respectively) and plasma lyso-Gb3 level were also normal (<1.00 ng/mL in both). However, she did not visit our hospital in person, and unfortunately the local hospital did not perform a urine test.

The patient already had advanced CKD at the time of diagnosis of Fabry disease, but had experienced stroke,

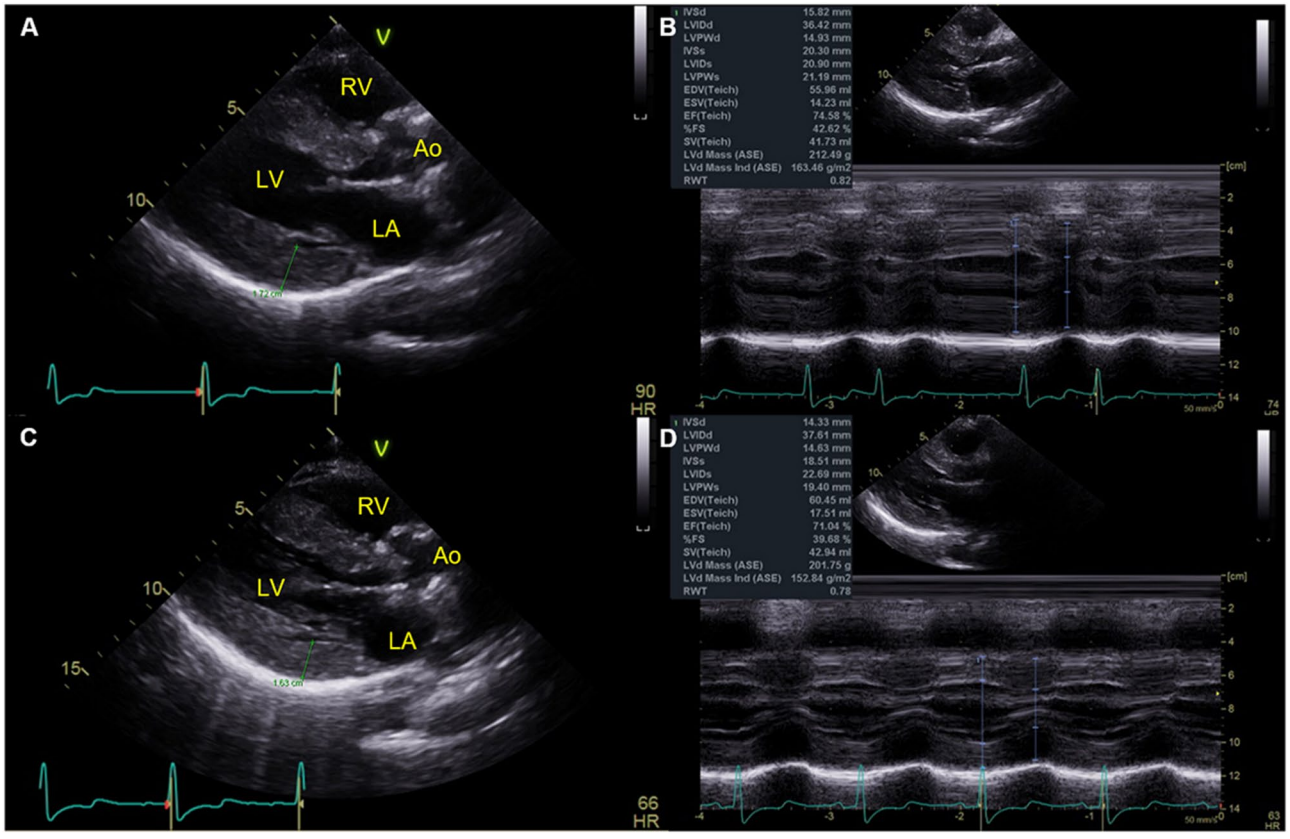

Fig. 1 Echocardiography before enzyme replacement therapy showed left ventricular hypertrophy with a posterior wall thickness of 17.2 mm in parasternal long axis view (**A**) and left ventricular mass index of 163.5 g/m^2 on M-mode echocardiography (**B**). Follow-up echocardiography after enzyme replacement therapy showed a mild regression of left ventricular hypertrophy with a posterior wall thickness of 16.3 mm (**C**) and left ventricular mass index of 152.8 g/m^2 (**D**). LV, left ventricle; RV, right ventricle; LA, left atrium; Ao, aorta

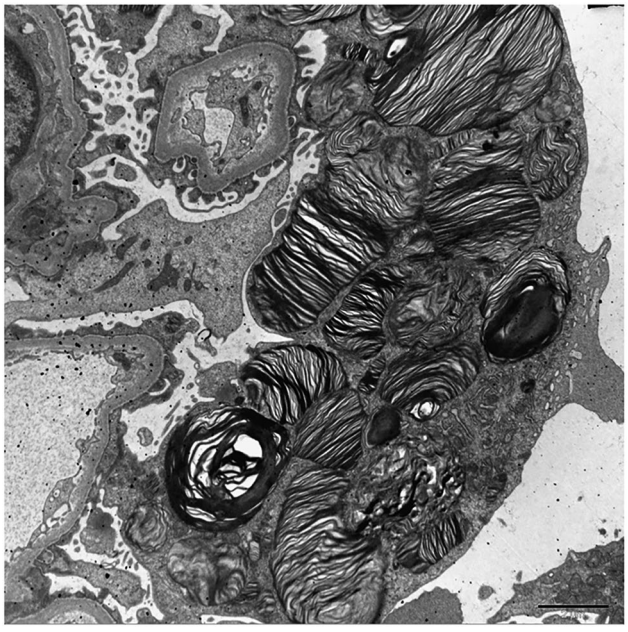

Fig. 2 Electron microscopy showed lamellated lipid inclusions in podocytes; the features were compatible with Fabry disease (original magnification × 8,000; bar = 2 μm)

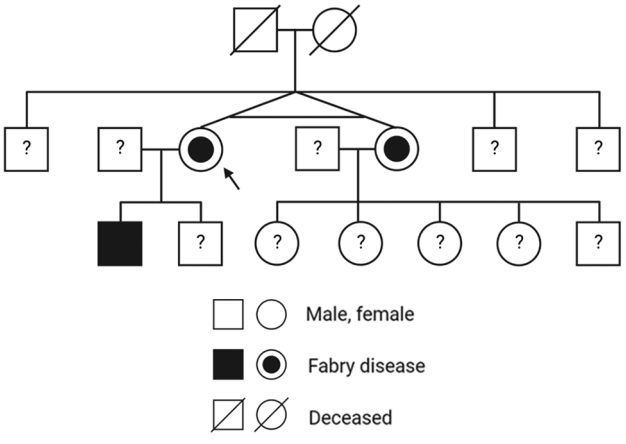

Fig. 3 Pedigree of the patient's family and complex intronic haplotype (c.−10 C > T, c.369 + 990 C > A, c.370–81_370–77delCAGCC, c.640–16 A > G, c.1000–22 C > T) carriers. An arrow indicates the index case

had cardiomegaly, and complained of persistent nonspecific abdominal pain. Therefore, we decided to start ERT with 1 mg/kg agalsidase-β every 2 weeks to prevent future cardiovascular complications and improve the patient's symptoms. Within the early period of ERT,

abdominal pain was substantially reduced. During the 2-year of follow-up with ERT, the level of eGFR decreased to 9.87 mL/min/1.73 m^2. Echocardiography showed no progression of left ventricular hypertrophy and a slightly decreased left ventricular mass index of 152.8 g/m^2 (Fig. 1C and D). We followed up the level of lyso-Gb3 every 4–6 months after starting ERT. But the level of lyso-Gb3 did not change and it stayed below the 1.00 ng/mL. However, her monozygotic twin sister refused ERT treatment due to the need for frequent intravenous injections. The follow-up echocardiography of her twin sister after 3 years showed little change in left ventricular hypertrophy from 115.5 to 118.0 g/m^2, and the eGFR was maintained at 51 mL/min/1.73m^2.

Discussion

Only a few cases of intronic haplotypes accompanied by classical symptoms of Fabry disease have been reported [6–8, 10, 12–15]. Fabry disease phenotype-related intronic haplotype variants are rare, and it is unclear whether intronic variants of the *GLA* gene correlate with Fabry disease. However, there are several ways that non-coding intronic variants could induce the clinical symptoms of Fabry disease. First, recent studies have demonstrated decreases in GLA enzyme expression in patients with the c.–10 C>T (rs2071225) variant located in the *GLA* promoter [6, 12, 15]. Similar to our case of CIH variants, mutation of the promoter region can decrease *GLA* expression, resulting in classic manifestations of Fabry disease [12, 13]. Second, intronic variations can affect alternative pre-mRNA splicing. Ishii et al. (10) showed that abnormal splicing in the mid-intronic *GLA* mutation of IVS4+919 A>G increased the alternatively spliced *α-galactosidase A* transcript, resulting in the Fabry cardiac phenotype. In fact, changes in *GLA* gene splicing were observed in the two intronic variants of c.370–81_370–77delCAGCC (rs5903184) and c.640–16 A>G (rs2071397) among the patients with CIH mutations [6]. Fabry disease has extensive phenotypic heterogeneity, even within patients with the same variants. The phenotype is probably modified not only by genetic factors but also epigenetics or factors unrelated to *GLA* [16]. The c.–10 C>T (rs2071397) variant is situated in a CpG island that contributes to epigenetic mechanisms by being the site of DNA methylation [17]. DNA methylation is a universal epigenetic mechanism for modifying gene expression. Thus, epigenetic changes may aberrantly reduce *GLA* gene expression when combined with this prevalent CIH sequence [15]. However, an additive effect of a number of intronic variants has been suggested by a previous report, although it was not conclusive [15].

Intron variants such as CIH variants may not be diagnosed using traditional exon-oriented sequencing. Our patient also had symptoms and clinical manifestations of Fabry disease but had difficulties in diagnosis. All the other GLA polymorphisms that constitute this CIH are relatively close to exon/intron boundaries, with the exception of c.369+990 C>A (rs1023431). Their detection in routine Sanger sequencing for the genetic diagnosis of Fabry disease depends on the position of the polymerase chain reaction (PCR) primers used. This variability in primer placement is one of the reasons why this CIH has been inconsistently described in the literature. In patients suspected of having a genetic disease due to a typical course or family history, but for whom an exonic mutation is not found, it may be necessary to perform intronic gene analysis. In recent studies, there have been reports of detecting novel pathogenic intronic variants using long-read sequencing [18]. If such techniques are introduced to diagnosis of Fabry disease, it will explain an important part of the missing heritability even in patients whose causative variants has not been diagnosed using methods such as Sanger sequencing or new generation sequencing. The different clinical course between the twin sisters in our case seems to be understandable in this context. Even if it is not limited to twins, intrafamilial variability is common in Fabry disease, and this is an important cause of delay in diagnosis and treatment of Fabry disease [19]. Therefore, following the diagnosis of Fabry disease, complete family screening is necessary, and it is important to observe the development of clinical symptoms through long-term follow-up.

There have been several reported cases of Fabry disease in female monozygotic twins. Discordant phenotypes in females with Fabry disease who have the same variants are explained by an imbalance in maternal and paternal X-chromosome expression. The separation of the fertilized egg into two monozygotes occurs before the second week of embryonic life, with X-chromosome inactivation happening around day 16, in a random basis [3]. Therefore, variable phenotypic expression can be observed in monozygotic female twins with X-linked disorder like Fabry disease [2–4]. In previously reported cases of female monozygotic twins, the twin sister of the proband typically did not develop clinical symptom of Fabry disease. However, in our case, the monozygotic twin sister of the proband exhibited similar clinical phenotypes, including cardiac, cerebrovascular, and kidney manifestations of Fabry disease. Furthermore, this is the first reported case caused by CIH variants.

ERT has long been the main therapeutic strategy for patients with Fabry disease. There have been several case reports of patients with CIH variants treated with ERT [7, 9, 12, 20]. A symptomatic German female −10T allele carrier started treatment with agalsidase-β, and this led to clinical stabilization of Fabry disease symptoms and a significant reduction in neuropathic

pain [9]. Interestingly, reducing the enzyme dose to half resulted in increased pain and reduced physical activity. In another study, two female Spanish patients with 5 CIH variants who presented with cerebrovascular disease and/or acroparesthesias achieved clinical stability after ERT [20]. Our index patient received ERT, while her twin sister did not. Two years of ERT may have stabilized the disease and reduced the abdominal pain in the patient. Therefore, it is necessary to observe how ERT or not having ERT will affect long-term prognoses in these monozygotic twins with the same genetic background. However, in terms of reduction in GFR, a decline in renal function was observed despite ERT, which is presumed to be due to the delayed diagnosis of Fabry disease in this patient and the delayed start of ERT. Wanner et al. reported that the lower baseline eGFR was associated with renal disease progression in women with Fabry disease [21]. Even in patients who underwent ERT, it was reported that the eGFR slope was steeper when baseline renal injury was severe [22]. Our patient had moderate-degree interstitial fibrosis and tubular atrophy observed in a renal biopsy 2 years before diagnosis, and it is presumed that her renal function deteriorated despite ERT. Lastly, the ERT period was relatively short in our patient, so the effect of ERT does not seem to differ dramatically between twins yet, but long-term follow-up is needed in the future.

Our study has limitations. First, we could not perform the functional study but there are some results of the previous studies which tested the functional effects of the CIH variants. Gervas-Arruga et al. reported that CIH carriers exhibited altered GLA expression, despite most carriers having high residual enzyme activity [6]. In addition, c.-10 C>T (rs2071225) variant which is located in the promotor region was shown to have decreased protein binding capacity in the EMSA study. Furthermore, Zeevi et al. reported that the patients with intronic variants demonstrated reduced mRNA expression of the GLA gene, suggesting a potential additive effect of these intronic variants [15]. We believe that these findings provide indirect evidence of the functional impact of CIH variants. Second, we were unable to confirm the nature of lamellated lipid inclusions in podocytes observed on EM. However, in previous studies, the accumulation of Gb3 was confirmed through methods such as anti-CD77 fluorescence in skin biopsy specimens of patients or in vitro studies with CIH variants [6, 8]. Third, despite the various evidence presented in this study and previous studies, it remains unclear whether CIH variants are truly pathogenic. Further research will likely be required, including the continuous accumulation of data on the relationship between intronic variants and phenotypes, as well as the application of new research techniques. Nevertheless, this is the first reported case of female monozygotic twins with CIH variants manifesting cardiac, cerebrovascular, and renal symptoms suggestive of Fabry disease. Our findings suggest that intronic gene analysis may be necessary in patients without exonic variants who exhibit Fabry disease symptoms.

Abbreviations

CIH	Complex Intronic Haplotype
eGFR	Estimated Glomerular Filtration Rate
ERT	Enzyme Replacement Therapy
Gb3	Globotriaosylceramide
Lyso-Gb3	Globotriaosylsphingosine
VUS	Variants of Uncertain Significance

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Author contributions

Conceptualization: CSK. Resources: SSK, JYC, CSK. Supervision: EHB, SKM, SWK, CSK. Writing – original draft: HSC, OIK, CSK. Writing - review & editing: HSC, OIK, SSK, JYC, EHB, SKM, SWK, CSK.

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Data availability

All data generated or analysed during this study are included in this published article.

Declarations

Ethical approval

This study was approved by Institutional Review Board of Chonnam National University Hospital (CNUH-EXP-2023-300). The patients/participants provided their written informed consent to participate in this study.

Consent for publication

Written informed consent was obtained from all the participants and parents/legal guardians and next of kin of the deceased participants for the publication of any potentially identifiable images or clinical details included in this study.

Competing interests

The authors declare no competing interests.

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